

**Supplemental Figure S2.** ValRS protein alignments and conservation analysis. (*A*) ValRS protein alignments, performed using NCBI COBALT (Papadopoulos and Agarwala 2007), from eight species indicated under the organism tab. One notable exception was that the ValRS from *Solanum tuberosum* represents the mitochondrial form, as the cytosolic enzyme sequence was not found. Red indicates strong conservation, blue indicates weaker conservation, and grey indicates gaps in conservation. (*B*) Example of the COBALT sequence alignment performed in panel A corresponding to the boxed region in panel A with each species indicated. (*C*) The structural model of *T. thermophilus* ValRS (light green) with conserved residue positions, by identity across all species, highlighted yellow, determined by Clustal Omega (Sievers et al. 2011; Sievers and Higgins 2018). (*D*) Structural alignment of ValRS from *T. thermophilus* (light green) with a model of ValRS from *S. tuberosum* generated using Phyre2 (Kelley et al. 2015) (orange). (*E,F*) The Phyre2 *S. tuberosum* structural model colored to match the conservation and alignment from panel A in the absence (E) and presence (F) of tRNA<sup>val</sup> (teal).